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pH Measurement of Mixed Saliva using a Flow-injection Sensor with a Tubular Carbon Electrode

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Present work discusses the design of a flow-injection sensor for measuring pH in micro-volumes of the sample. A carbon electrode with a capillary channel was used as a working electrode, through which an electrolyte solution saturated with quinhydrone flows at a constant speed. The volume of the analyzed sample was 0.1-0.2 mL. Compliance with the Nernst pH function with a slope of 53.1 ± 1.1 mV/pH was observed in the range of 1.0-7.5 pH. The sensor has been tested in the non-invasive diagnostics of acid-base balance of the oral fluid. The analysis of mixed saliva was carried out in 30 practically healthy student volunteers with an intact row of teeth. The median pH value of 7.03 was established, which corresponds to the physiological norm. At the same time, there is a predisposition to caries in almost half of the subjects, since a high risk is determined in the formation of focal demineralization of tooth enamel with the appearance of erosion of hard tooth tissues.

Keywords: Non-invasive diagnostics, Mixed saliva, pH determination, Flow-injection sensor, Quinhydrone electrode.

INTRODUCTION

One of the most preferred objects of non-invasive monitoring of the functional state of the body may be the fluid of the oral cavity. It is known that oral fluids are produced by three pairs of salivary glands, each of which produces its characteristic fluids: serous or parotid, serous-mucous and sublingual-mucous [1]. The sources affecting the composition of the oral fluid are the gingival slits and salivary channels (located on the tongue, buccal mucosa and palate) and the nasopharynx [2,3].

Due to such a variety of salivary fluid, the analysis of mixed saliva samples is the most preferable, since it minimizes these differences in composition. Mixed saliva is a viscous liquid with a pH of 5.8-7.6, the composition varies depending on the rate of its secretion. Physiologists have found that a healthy person produces 500 to 1500 mL of saliva during the day. The main component of saliva is water (up to 98%) in which mineral and organic substances are dissolved [4]. Most of the electrolytes in saliva are sodium, potassium, calcium cations and anions in the form of chloride, bicarbonate and hydrophosphate ions [5-7], which are secreted by salivary glands from blood plasma. Trace elements Fe, Si, Mn, Ni, Li, Zn, Cd, Pb, Li, *etc.* are also found in saliva. All mineral macro- and microelements are found both in the form of simple ions and in the composition of compounds *e.g.* salts, proteins and chelates, *i.e.* all substances present in whole blood and its plasma [8-10]. But this does not mean that saliva is a complete copy of plasma. It has its own characteristics, chemical and biochemical properties.

At the same time, correlations were found between the content of elements in saliva and in other bioassays, in particular Cd in hair (p = 0.201 $p < 0.01$), Cr in urine (p = 0.179, $p < 0.05$), Ni in hair (p = 0.248, $p < 0.05$) [11], Hg in hair (p = 0.887, $p < 0.001$) [12], Mn (r = 0.545 $p < 0.05$) and Cu (r = 0.504 $p < 0.05$) in blood serum [13].

The composition of saliva is influenced by its circadian rhythm, which determines the volume of saliva and its quantitative and qualitative composition, as well as age, nutrition system, oral hygiene, hormonal changes. As a result, the composition of saliva changes both quantitatively and qualitatively, which causes a fundamental difference from blood plasma.

The composition analysis of mixed saliva for the corresponding biomarkers of a number of diseases and pathologies can open up new possibilities for its use in clinical diagnostics [14]. Thus, it has been established with high probability that some systemic diseases, medications and psychotropic substances affecting the central and peripheral nervous systems, as well as an unfavourable environment, are able to change the composition of saliva [15].

Thus, saliva, as an object of non-invasive monitoring, reflects the level of substances injected into the body and can be an indicator of emotional, hormonal, immunological, neurological status, nutrition and metabolic disorders. Saliva, as an object of research, can also be used in the diagnosis of oral and systemic diseases associated with the salivary glands, for example, Sjogren's syndrome [16], benign and malignant tumors of the oral cavity, periodontal diseases, caries and has become a useful tool for studying some of the main problems in the field of physiology [16,17]. Thus, complex saliva analysis can play an important role for the detection of pathologies and diagnosis of systemic diseases, being an additional (and sometimes alternative) source of medical information that increases the accuracy of diagnosis [18-20]. Thus, the determination of heavy metals in saliva can be used in monitoring the influence of the environment and other specific pollutants that lead to occupational diseases [10-12].

The advantages of saliva analysis in comparison with other bioassays are the complete noninvasiveness of sampling and its simplicity, which does not require certain skills of a laboratory assistant, the possibility of multiple sampling, which allows monitoring, lower requirements for the storage and transportation of saliva samples, as well as the absence of coagulability characteristic of blood [21-25].

The pH value of mixed saliva is an objective criterion for assessing the state of the acid-base balance responsible for the biochemical processes occurring in the human oral cavity and determining the formation of dental deposits, the re- and demineralization of tooth enamel, the life activity of the oral microflora, *etc.* [26]. According to the hydrogen indicator value and its change in saliva, it is possible to assess the state of local homeostasis [27]. Several researchers devoted their study on the activity of hydrogen ions as indicator in the oral cavity [28-31]. This interest is due to a number of factors, such as the possibility of simply establishing the slightest disturbances of acid-base balance in the oral cavity, occurring under the influence of physiological and pathological processes. The pH shift can be caused by alimentary factors, occupational and environmental hazards, the use of hygiene products and medicines, prostheses, fillings, the state of the macroorganism [32]. The most relevant is the study of the regulation of the key-base balance in the oral cavity in terms of early diagnosis and prognosis of dental diseases: dental caries, inflammatory periodontal diseases.

Among the analytical methods of pH measurement, the method of direct potentiometry with the use of glass or antimony pH-sensitive electrodes is widely used [33]. In laboratory practice, glass electrodes are usually used to measure pH. But it should be noted that the geometric dimensions of such electrodes require large volumes of the analyzed liquid and mixing. The problem is not solved even with the use of microcells included in the equipment of the devices. However, there is a known method for determining the pH using a so-called quinhydrone electrode, which is a platinum wire in contact with the analyzed solution saturated with quinhydrone [33]. Theoretical ideas about the principle of functioning of the hingidron electrode and the limitations of its use in analysis are also described [33], as well as the possibility of pH measurement with a carbon electrode modified with a quinhydrone [34]. But even here, the problem of the volume of the analyte remains.

In our opinion, the use of flow-injection potentiometric analysis using a tubular carbon electrode, through the channel of which an electrolyte saturated with quinhydrone flows at a constant rate, will solve this engineering problem. The design of such a sensor and its approbation in determining the pH of saliva determined the purpose of this work.

EXPERIMENTAL

The object of the analysis was samples of mixed saliva collected from 30 volunteer donors (1-2 year undergraduate students of the Faculty of Chemistry of the Samarkand State University). All volunteers who participated in the study gave their informed consent and were told of the purpose of the study.

Sample collection: Sampling was carried out from 12 to 14 h in order to minimize circadian changes in the composition of saliva. The volunteers did not take food for at least an hour and a half before sampling. Before direct sampling, donors rinsed the oral cavity with distilled water. No stimulation was performed when collecting saliva. The saliva formed during the first 2 min after rinsing the oral cavity was not used for analysis and was spat out. Collection of the following portions of saliva were collected in plastic tubes with a volume of 3 mL. The contents of the test tubes were centrifuged at 6000 rpm for 15 min.

The working solution was a saturated solution of quinhydrone in double-distilled water, which was fed into the channel of the tubular carbon electrode of sensor using a peristaltic pump at a rate of 1.0- 1.5 mL/min. For the manufacture of a measuring pH-sensitive electrode, carbon graphite powder with particle sizes ~0.1 was used, obtained by grinding carbon electrodes for spectral analysis corresponding to TU 01-7-67 in an electric mill. The resulting powder was introduced in small portions into the melted paraffin in a ratio of 50:50 with constant stirring with a glass rod. The molten mixture was loaded into thin-walled copper tubes with a diameter of 6 mm and a length of 30 mm, pre-cleaned and treated with a solution of nitric acid, followed by washing with distilled water. After solidification of the carbon-paraffin mixture and its "aging" for 24-48 h, a channel with a diameter of 1 mm was drilled along the entire length of the electrode blank, which was polished with a cotton thread passed through it.

The obtained electrodes were used in a flow sensor paired with a AgCl reference electrode. The electrochemical flow-injection sensor was calibrated according to standard buffer solutions prepared from fix channels of standard titers for pH measurement according to GOST 8.134-98.

With the help of an automatic pipette-type dispenser, 0.1 mL of measured samples were injected. The analytical signal was recorded in the form of peaks on the diagram tape of the recording potentiometer.

RESULTS AND DISCUSSION

The pH value can be used to judge the level of demineralization of tooth tissues. Saliva constantly maintains a neutral reaction, as it has a pronounced buffer capacity due to the phosphates and proteins dissolved in it. Modern studies show that the prerequisite for caries is the long-term effect of acids on tooth tissue. With a decrease in pH, saliva (protein part of the organic fraction) retains free calcium, which contributes to the demineralization of hard tooth tissues. At the same time, saliva is a biological fluid oversaturated with hydroxyapatite and this prevents the dissolution of enamel and promotes the diffusion of calcium and phosphorus ions into the enamel. The degree of oversaturation is greatest in people with a high level of caries resistance. However, such processes can be observed only up to pH = 6.0-6.2 (critical level) and with further acidification, saliva turns from a remineralizing to a demineralizing liquid. These arguments once again emphasized the relevance of determining the pH in saliva for early diagnosis of the acid-base balance of oral fluid.

An electrochemically reversible redox quinone-hydroquinone pair is used to determine the pH of solutions by the method of a quinhydrone electrode, the ratio of oxidized and reduced forms of which depends on the activity of hydrogen ions in the analyzed solution. To measure the pH in this case, a small amount of quinhydrone is injected into the analyzed solution, which is an equimolecular mixture of quinone and hydroquinone (1:1). The EMF of the created galvanic cell is reduced between the platinum wire and the silver chloride as reference electrode immersed in the analyzed solution. The disadvantages of such a measuring system include the limited range of pH determination (< 8), due to the fact that hydroquinone decomposes in an alkaline medium, as well as some inconveniences associated with the operation of such an electrode during routine measurements.

At the same time, the pH of the oral fluid is in the range of 4-7, which determines in advance the limit of the operational capabilities of the quinhydrone electrode. It remains up to the "small" – to reduce the volume of the analyzed liquid. This goal is achieved by using a microcell, which is a capillary made of an inert conductive material, *i.e.* graphite. Also, the design of the sensor should be such that it is possible to make contact between the working tubular electrode and the reference electrode, which is used as a silver chloride electrode.

Fig. 1 shows one of the variants of an electrochemical sensor, the principal possibility of using it to determine the pH in micro-volumes of the sample, has to be proved. The pH-metric sensor consists of a housing made of transparent organic glass, contains a measuring tubular electrode with a capillary channel with a diameter of 1 mm and a length of 30 mm; a comparative silver chloride electrode of the EVL-1M or EVL-4M type located in a micro-chamber in contact with the measuring tubular electrode by means of a 0.1 M KCl electrolyte solution saturated with quinhydrone; it has a fitting for supplying an electrolyte solution supplied by a peristaltic pump to the measuring volume and a valve for entering the liquid to be disposed of, the pH of which is to be measured. It should be observed that the design features of sensor allow for amperometric measurements in galvanostatic mode [35]. The contacts of the electrodes were connected to a pH meter connected to a writing potentiometer or other analog-to-digital converter.

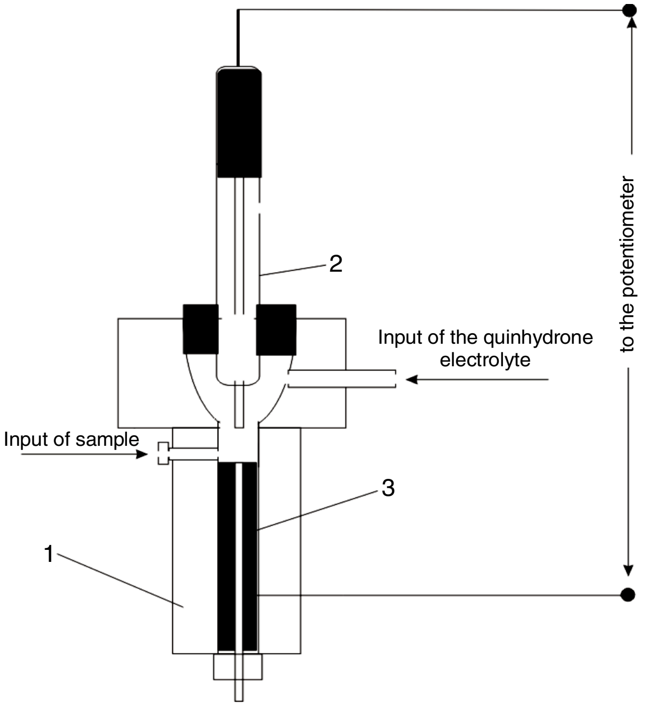

Fig. 1. Schematic diagram of a flow-injection sensor for pH measurement [1 = housing; 2 = silver chloride reference electrode; 3 = tubular carbon electrode]

Previously, the sensor was calibrated according to standard sample solutions for pH measurement, for which 100 mL of solutions were injected into the electrochemical sensor by means of a valve. The analytical signal was recorded on a self-recording potentiometer in the form of peaks. The interval between measurements does not exceed 1 min, which allows up to 60 measurements per hour.

During this experiment, it was found that the analytical signal (Fig. 2) adequately notes the change in the pH of the solution, with the steepness of pH function - 53.2 ± 1.1 mV/pH and is linear in the entire measured range, represented by exemplary reference solutions of 1.68 - 6.86 pH. When moving to a more alkaline region in the range of 8.0-9.2 pH, the slope of the calibration curve is ~ -47.5 mV/pH (Fig. 3).

After calibration of the sensor, measurements were made of the saliva pH of the examined volunteers. Since all the subjects had a complete set of teeth, it can be assumed that the experiment was conducted with intact teeth. For the analysis, a transparent supravent fluid obtained after centrifugation of the collected mixed saliva was used. The results of the experiment are presented in Table-1. The arithmetic mean was 6.952 and the median pH of the mixed saliva of practically healthy people was 7.03.

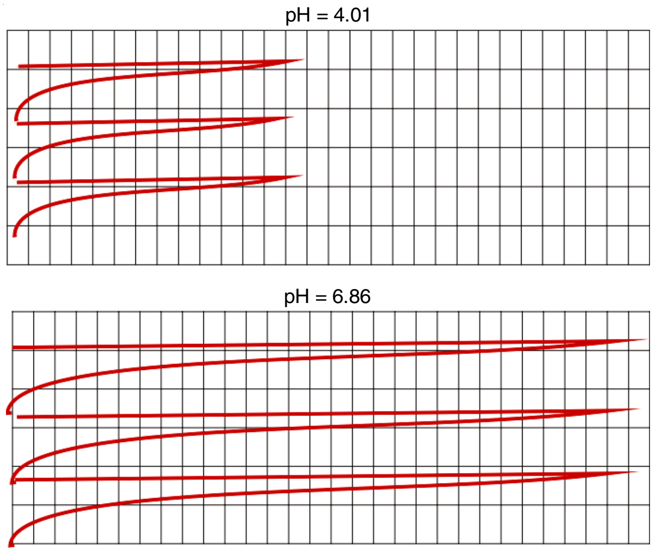

Fig. 2. The form of recording the analytical signal

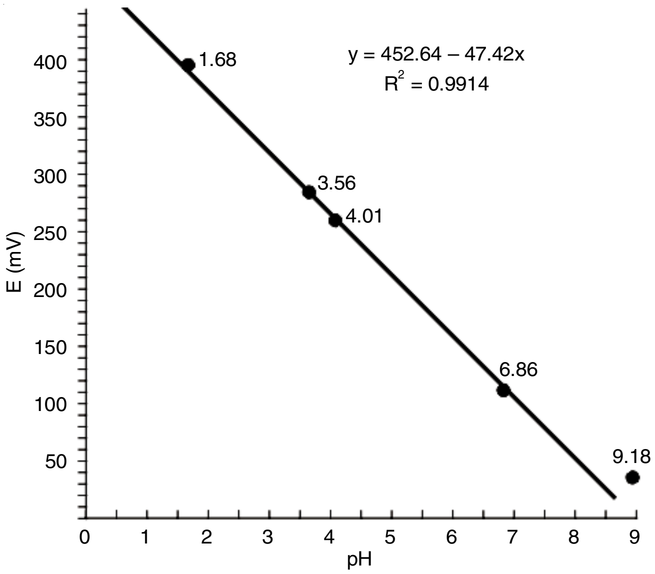

Fig. 3. Calibration dependence of the flow-injection quinhydrone electrode

TABLE-1
DETERMINATION OF pH IN SALIVA OF HEALTHY TEST VOLUNTEERS USING A FLOW-INJECTION POTENTIOMETRIC SENSOR WITH A HINGIDRON ELECTRODE

Sample	pH	Sample	pH	Sample	pH
1	6.82	11	6.24	21	7.0
2	7.21	12	7.14	22	6.86
3	6.68	13	7.11	23	7.62
4	7.05	14	6.86	24	7.32
5	6.15	15	7.48	25	5.92
6	6.96	16	6.92	26	7.16
7	7.18	17	6.32	27	7.20
8	7.14	18	7.28	28	7.22
9	6.87	19	6.54	29	7.04
10	7.02	20	7.18	30	6.98

In 17 people examined (~ 57%) pH > 7, which corresponds to the norm usually accepted in dentistry; in 9 people, the saliva pH is less than 7, but does not reach the critical value of 6.5, which is 30% of the examined; for 4 people (13%), the saliva pH value is in the "critical" zone, *i.e.* < 6.3-6.2. For one subject, a 20-year-old girl wearing braces, the saliva pH showed the lowest (acidic) indicator -5.92, which makes one think about the question: how braces and their composition can affect the acid-base processes occurring in the oral cavity cavities?

Conclusions

A flow-injection type potentiometric sensor was prepared with a working tubular pH sensitive electrode made of spectrally pure coal with dimensions of 6 mm × 30 mm with a capillary channel with a diameter of 1 mm, through which an electrolyte saturated with quinhydrone flows at a constant speed of 60-90 cm^3/h. The steepness of the electrode pH function is close to the Nernst one and is -53.2 ± 1.1 mV/pH. The sensor has been tested successfully in determining the pH of mixed saliva. In the course of a non-invasive study, it was revealed that despite a comparatively good level of organization of therapeutic and preventive work, a predisposition to caries is observed in almost half of the examined student volunteers, while a high risk is determined in the formation of focal demineralization of tooth enamel with the appearance of erosion of hard tooth tissues and the formation of caries in them. For a group of subjects with a critical pH value (6.2-6.5), it is recommended to undergo a preventive examination at the dentist and as an effective measure, to exclude the consumption of carbonated beverages, to rinse the mouth with water at night with the addition of baking soda. These measures, in our opinion, will help to preserve an intact row of teeth.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. J.M. Lee, E. Garon and D.T. Wong, *Dent. Abstr.*, **56**, 53 (2011); https://doi.org/10.1016/j.denabs.2009.10.037
2. J.K.M. Aps and L.C. Martens, *Forensic Sci. Int.*, **150**, 119 (2005); https://doi.org/10.1016/j.forsciint.2004.10.026
3. D.J. Crouch, *Forensic Sci. Int.*, **150**, 165 (2005); https://doi.org/10.1016/j.forsciint.2005.02.028
4. A.Z. Reznick, N. Shehadeh, Y. Shafir and R.M. Nagler, *Arch. Oral Biol.*, **51**, 640 (2006); https://doi.org/10.1016/j.archoralbio.2006.02.004
5. L. Heflin, S. Walsh and M. Bagajewicz, *Comput. Chem. Eng.*, **33**, 1067 (2009); https://doi.org/10.1016/j.compchemeng.2008.09.024
6. S. Chiappin, G. Antonelli, R. Gatti and E.F. De Palo, *Clin. Chim. Acta*, **383**, 30 (2007); https://doi.org/10.1016/j.cca.2007.04.011
7. M. Esteban and A. Castano, *Environ. Int.*, **35**, 438 (2009); https://doi.org/10.1016/j.envint.2008.09.003
8. I. Kocadereli, A. Ataç, S. Kale and D. Özer, *Angle Orthod.*, **70**, 431 (2000); https://doi.org/10.1043/0003-3219(2000)070<0431:SNACIP>2.0.CO;2
9. I.D. Mandel, *J. Oral Pathol. Med.*, **19**, 119 (1990); https://doi.org/10.1111/j.1600-0714.1990.tb00809.x
10. D.K. Lloyd, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **866**, 154 (2008); https://doi.org/10.1016/j.jchromb.2008.01.058
11. F. Gil, A.F. Hernández, C. Márquez, P. Femia, P. Olmedo, O. López-Guarnido and A. Pla, *Sci. Total Environ.*, **409**, 1172 (2011); https://doi.org/10.1016/j.scitotenv.2010.11.033

12. H. Fakour, A. Esmaili-Sari and F. Zayeri, *J. Hazard. Mater.*, **177**, 109 (2010); https://doi.org/10.1016/j.jhazmat.2009.12.002
13. D. Wang, X. Du and W. Zheng, *Toxicol. Lett.*, **176**, 40 (2008); https://doi.org/10.1016/j.toxlet.2007.10.003
14. Y. Cui, M. Yang, J. Zhu, H. Zhang, Z. Duan, S. Wang, Z. Liao and W. Liu, *Med. Novel Technol. Dev*., **13**, 100115 (2022); https://doi.org/10.1016/j.medntd.2022.100115
15. R. Pramanik, H. Thompson, J.O. Kistler, W.G. Wade, J. Galloway, T. Peakman and G.B. Proctor, *Int. J. Epidemiol.*, **41**, 1786 (2012); https://doi.org/10.1093/ije/dys166
16. V.C. Romão, R. Talarico, C.A. Scirè, A. Vieira, T. Alexander, C. Baldini, J.-E. Gottenberg, H. Gruner, E. Hachulla, L. Mouthon, M. Orlandi, C. Pamfil, M.P. de Chambrun, M. Taglietti, N. Toplak, P. van Daele, J.M. van Laar, S. Bombardieri, M. Schneider, V. Smith, M. Cutolo, M. Mosca and X. Mariette, *RMD Open*, **4(Suppl 1)**, e000789 (2018); https://doi.org/10.1136/rmdopen-2018-000789
17. M.A. Javaid, A.S. Ahmed, R. Durand and S.D. Trand, *J. Oral Biol. Craniofac. Res.*, **6**, 66 (2016); https://doi.org/10.1016/j.jobcr.2015.08.006
18. L. Vitali, V.T. Fávere and G.A. Micke, *J. Chromatogr. A*, **1218**, 2327 (2011); https://doi.org/10.1016/j.chroma.2011.02.035
19. C.Z. Zhang, X.Q. Cheng, J.Y. Li, P. Zhang, P. Yi, X. Xu and X.-D. Zhou, *Int. J. Oral Sci.*, **8**, 133 (2016); https://doi.org/10.1038/ijos.2016.38
20. M.L. Hartman, F. Groppo, M. Ohnishi, J.M. Goodson, H. Hasturk, M. Tavares, T. Yaskell, C. Floros, K. Behbehani and M.S. Razzaque, *Contrib. Nephrol.*, **180**, 138 (2013); https://doi.org/10.1159/000346793
21. V.V. Khudoley and I.V. Mizgirev. Environmentally Hazardous Factors, St. Petersburg: Petrovsky Bank, p. 186 (1996).
22. W. Zheng, S.X. Fu, U. Dydak and D.M. Cowan, *Neurotoxicology*, **32**, 1 (2011); https://doi.org/10.1016/j.neuro.2010.10.002
23. J.P. Bonde and J.M. Christensen, *Arch. Environ. Health*, **46**, 225 (1991); https://doi.org/10.1080/00039896.1991.9937453
24. C. Timchalk, T.S. Poet, A.A. Kousba, J.A. Campbell and Y. Lin, *J. Toxicol. Environ. Health*, **67**, 635 (2004); https://doi.org/10.1080/15287390490428035
25. N. Jacobs, N.A. Nicolson, C. Derom, P. Delespaul, J. van Os and I. Myin-Germeys, *Life Sci.*, **76**, 2431 (2005); https://doi.org/10.1016/j.lfs.2004.10.045
26. V.K. Leontiev, *Dentistry*, **78**, 22 (1999).
27. A.I. Volozhin, I.V. Alekseeva, A.Zh. Petrikas and V.A. Rumyantsev, Pathophysiology of Acid-Base Equilibrium, Mir Pubilshers: Moscow, p. 60 (1991).
28. K.E. Kaczor-Urbanowicz, C. Martin Carreras-Presas, K. Aro, M. Tu, F. Garcia-Godoy and D.T.W. Wong, *Exp. Biol. Med.*, **242**, 459 (2017); https://doi.org/10.1177/1535370216681550
29. M. Mal, *Future Sci. OA*, **2**, FSO106 (2016); https://doi.org/10.4155/fsoa-2015-0014
30. R.S. Malon, S. Sadir, M. Balakrishnan and E.P. Córcoles, *BioMed Res. Int.*, **2014**, 962903 (2014); https://doi.org/10.1155/2014/962903
31. S. Baliga, S. Muglikar and R. Kale, *J. Indian Soc. Periodontol.*, **17**, 461 (2013); https://doi.org/10.4103/0972-124X.118317
32. P.N. Galgut, *J. Int. Acad. Periodontol.*, **3**, 61 (2001).
33. B. Kumar, N. Kashyap, A. Avinash, R. Chevvuri, M.K. Sagar and K. Shrikant, *Int. J. Contemp. Dental Med. Rev.*, **2017**, 011217 (2017).
34. S.D. Aronbayev, D. Aronbayev, H. Xolmirzaeva, D. Isakova and G. Narmaeva, *Universum: Chem. Biol.*, **2**, 21 (2020).
35. A.D. Markielovich and V.S. Mikhailovna, *Young Scientist*, **21**, 28 (2015).